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CHANGES IN THE AMOUNT OF DIFFUSIBLE AUXIN AND THE ACTIVITY OF IAA-OXIDASE FRACTIONS ON THE EFFECT OF 2-CHLOROETHYL-TRIMETHYL-AMMONIUM CHLORIDE (CCC)

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Abstract

In bean plants the content of diffusible IAA increased on the effect of a treatment with CCC, indicating that the auxin supply of the tissues in the elongation zone did not decrease on the effect of the retardant. The increase of the amount of diffusible IAA may be correlated with the effect of CCC on the permeability of the membrane. The increase of activity of the ionically wall-bound IAA-oxidase measurable on the effect of the treatment is playing a definite role in the limitation of elongation.

Introduction

The observations according to which the endogenous IAA content of the plants decreased on the effect of a CCC-treatment (KURAISHI and MUIR, 1963; NORRIS, 1966; VOLYNETZ and PALCHENKO, 1977) fitted well into the concept developed for the mechanism of effect of the growth retardants.

However, in the regulation of growth in the first line not the total IAA content of the plants but rather the IAA-supply of the tissues present in the elongation zone is the factor of importance which is correlated also with the movement of auxin within the plant.

Informations concerning the latter cannot be obtained by the measurement of the total IAA content extractable from the homogenizate of the plants, particularly in case of a treatment with compounds such as CCC which causes a significant change in the permeability of the tissues (FABIJAN et al., 1981) and thus presumably also in the movement of auxin.

Materials and methods

For our investigations we used bean cultivars of various growth intensities (*Phaseolus vulgaris convar. vulgaris cv. Juliska and convar. nanus cv. Cherokee*). The seeds were swollen in a CCC (Merck-Schuchardt) solution of 1000 mg/l concentration in a 25 °C thermostat, then sown in garden mould and grown under controlled conditions.

The content of diffusible auxin was determined in the shoots above the cotyledone of plants aged 9 days by diffusion accelerated by centrifugation as modified according to the method of GOLDSCMIDT and MONSELISE (1968). The excised shoots were covered in a vertical position with 20% methanol and centrifuged 45 minutes at 1500 g. The diffusate was evaporated under reduced pressure to an aqueous residue and a 0.5 M solution of K_2HPO_4 was added to it (pH 8.5). The purification and fractionation were carried out according to the method of KNEGT and BRUINSMA (1973), further of HEMBERG and TILLBERG (1980). The indole compounds present in the final ethereal fraction were separated by TLC, using a solvent system of chloroform: ethyl acetate: formic acid (4:5:1). The amount of IAA was determined with a SPEKORD UV/VIS photometer, on applying the method of (FLETSCHER and ZALIK (1964).

IAA-oxidase preparation: 10 g epicotyl was ground with a double amount of phosphate buffer pH 7.2) and centrifuged 5 minutes at 1000 g. The supernatant was again centrifuged 20 minutes at 20 000 g. The supernatant obtained in this way represented the soluble fraction and the residue the membrane fraction (DARIMONT et al., 1977). The residue obtained at the first centrifugation was incubated 12 hours with a 0.3 M NaCl solution (SÁGI, 1979) and subsequently centrifuged 15 minutes at 12 000 g. The supernatant contains the ionically bound wall-peroxidases. The covalently bound fraction was obtained by the method of DARIMONT et al., (1973), by the treatment with pectinase (SIGMA) and cellulase (MERCK).

Polyclar-AT was used at the preparations. The protein content was measured according to the method of LOWRY et al., (1951).

IAA-oxidase assay: the amount of decomposed IAA was determined by photometry, using the method of GALSTON and DALBERG (1954), further of HILLMANN and GALSTON (1956). The reaction mixtures contained besides the enzyme extract and IAA also $MnCl_2$, DCP and H_2O_2. The samples were incubated 60 minutes at 30°C then 2 ml of GORDON-WEBER reagent was added to 1 ml of the sample and the developed colour determined after 30 minutes by photometry at 530 nm.

Results and discussion

On the effect a treatment with CCC the amount of diffusible auxin increased in comparison to those of the controls, both in case of normal and of dwarf beans (Table 1). The results indicate that the decrease of the extractable auxin content

Table 1. Effect of CCC on the amount of diffusible IAA

	diffusible IAA ng/ 100 plant	
	control	treated
cv. Juliska	40	49
cv. Cherokee	31	43

(KURAISHI and MUIR, 1963; VOLYNETZ and PALCHENKO, 1977) does not mean at the same time a weaker supply of auxin of the tissues present in the elongation zone, and they are calling our attention to the fact that owing to the increase of membrane permeability (FABIJAN et al., 1981) the auxin supply in the treated plants may be even better than that of the controls.

The effect of the treatment with CCC on the fractions of IAA-oxidase is shown by the data of Table 2. An increase of activity was experienced in the soluble and in the ionically wall-bound fractions.

Table 2. Effect of CCC on the fractions of IAA-oxidase

		IAA destroyed ($\mu g . mg^{-1}$ protein h^{-1})			
		soluble	membrane	wall-bound	
				ionic	covalent
cv. Juliska	control	12	13	22	12
	treated	16	14	38	12
cv. Cherokee	control	13	12	25	16
	treated	17	13	31	15

The increase of the activity of cytoplasmatic IAA-oxidase on the effect of CCC has been indicated also by other authors (HALEVY, 1963; EL-FOULY and JUNG, 1965; GASPAR and LACOPPE, 1968). However, from the aspect of a more direct correlation between the extension growth and the IAA-oxidase, taking into account the changes in the ultrastructure of the cell wall, rather the change of the activity of IAA-oxidase bound ionically to the cell wall is more worthy of attention.

The activity of the ionically wall-bound IAA-oxidase was determined also in the non-growing epicotyls and it was found that in the non-growing epicotyls of untreated plants the activity of this IAA-oxidase fraction was higher than in the growing epicotyl (destroyed IAA 30 $\mu g \cdot mg^{-1}$ protein $\cdot h^{-1}$). Therefore we presume that the ionically wall-bound IAA-oxidase is playing a definite role instead of the control of the rate of elongation, rather in its limitation and its conversion to irreversibility. Similar results were obtained by SÁGI (1979) in the case of lupin. The IAA-oxidase of the cell wall participates in the synthesis of lignin (STAFFORD, 1965; HARKIN and OBST, 1973) whereas the accumulation of lignin may be one of the causes of the discontinuance of growth WHITMORE, 1971). Thus, in the development of the retarding effect of CCC a significance must be ascribed also to the increased lignin synthesis.

References

DARIMONT, E., SCHACHOFER, K. and GASPAR, T. (1973): Isoperoxidases et hydroxyproline dans le parois cellulaires des racines de Lentille. — Biochim. Biophys. Acta *321*, 461—466.

DARIMONT, E., PENEL, C., AUDERSET, G., GREPPIN, H. and GASPAR, T. (1977): Peroxydases de haut poids moleculaire identifiées a des peroxydases membraire shez la Lentille. — Arch. Int. Physiol. Biochim. *85*, 497—507.

EL-FOULY, M. and JUNG, J. (1966): Anderung der Peroxidaseaktivität von Weizenpflanzen nach Behandlung mit CCC und Gibberellinsäure. — Naturwiss. *53*, 586—587.

FABIJAN, D. M., DHINDSA, P. P. and REID, D. M. (1981): Effects of two growth retardants on tissue permeability in *Pisum sativum* and *Beta vulgaris*. — Planta *152*, 481—486.

FLETCHER, R. A. and ZALIK, D. (1964): Effect of light quality on growth and free indoleacetic acid content in *Phaseolus vulgaris*. — Plant Physiol. *39*, 328—331.

GALSTON, A. W. and DALBERG, L. Y. (1954): The adaptive formation and physiological significance of indole-acetic oxidase. — Amer. J. Bot. *41*, 373—380.

GASPAR, T. and LACOPPE, J. (1968): The effect of CCC and AMO-1618 on growth, catalase, peroxidase and indoleacetic acid oxidase activity of young barley seedlings. — Physiol. Plant. *21*, 1104—1109.

GOLDSCHMIDT, E. E. and MONSELISE, S. P. (1968): Extraction of auxin and other growth regulators from citrus stems by centrifugal force. — Physiol. Plant. *21*, 754—758.

HALEVY, A. H. (1963): Interaction of growth-retarding compounds and gibberellin on indoleacetic acid oxidase and peroxidase of cucumber seedlings. — Plant Physiol. *38*, 731—737.

HARKIN, J. M. and OBST, J. R. (1973): Lignification in trees: indication of exclusive peroxidase participation. — Science *180*, 296—298.

HEMBERG T. and TILLBERG, E. (1980): The influence of the extraction procedure on yield of indole-3-acetic acid in plant extracts. — Physiol. Plant. *50*, 176—182.

HILLMANN, W. S. and GALSTON, A. W. (1956): Interaction of manganese and 2,4-dichlorphenol in the enzymatic destruction of indoleacetic acid. — Physiol. Plant. *9*, 230—235.

KNEGT, E. and BRUINSMA, J. (1973): A rapid, sensitive and accurate determination of indolyl-3-acetic acid. — Phytochem. *12*, 753—756.

KURAISHI, S. and MUIR, R. M. (1963): Mode of action of growth retarding chemicals. — Plant Physiol. *38*, 19—24.

LOWRY, R. H., ROSEBROUGH, N. J., FARR, H. L. and RANDALL, R. J. (1951): Protein measurement with the folinphenol-reagent. — J. Biol. Chem. *193*, 265—275.

NORRIS, R. F. (1966): Effect of CCC on the level of endogenous indole compounds in wheat seedlings. — Can. J. Bot. *44*, 675—684.

SÁGI, F. (1979): Az indolecetsav-oxidáz enzim sejten belüli megoszlása és növekedésélettani szerepe — Kandidátusi értekezés, Szeged. (Distribution of IAA-oxidase and its physiological role. Ph. D. Thesis, Szeged)

STAFFORD, H. A. (1965): Factors controlling the synthesis of natural and induced lignins in Phleum and Elodea. — Plant Physiol. *40*, 844—851.

WHITMORE, F. W. (1971): Lignin formation in wheat coleoptile cell walls, a possible limitation of cell growth — Plant Physiol. *48*, 596—602.

VOLYNETZ, A. P. and PALCHENKO, L. A. (1977): Chlorocholine chloride as a possible inhibitor of auxin biosynthesis and a factor limiting its utilisation in growth processes. — Fiziol. Rast. *5*, 1021—1025.

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